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## The effect of petroleum hydrocarbons concentration on competition between oil-degrading bacteria and indigenous compost microorganisms in petroleum sludge bioremediation

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### ARTICLE INFO

#### Article history:

Received 27 March 2021

Received in revised form 15 December 2021

Accepted 10 January 2022

Available online 17 January 2022

#### Keywords:

Bioremediation

Finished compost microorganisms

Oil-degrading bacteria

Petroleum sludge

### ABSTRACT

The influence of the concentration of total petroleum hydrocarbons (TPHs) on competition between isolated oil-degrading bacteria (ODB) and finished compost microorganisms (FCM) was investigated in composting bioreactors over 12 weeks period. First, the batch tests were performed in Bushnell–Haas medium (BHM) to evaluate the biodegradation activities of six native ODB isolated from petroleum sludge (PS). Then, the ODB were added to the composting bioreactors containing 10 and 30 g/kg of TPHs. Based on the BHM results, the highest degradation efficiency of crude oil (1%–3% concentration) were determined to be 72%–75% at pH 7 after 7 days. In the composting bioreactors containing only the ODB, TPHs removal rates were 86%–92% after 12 weeks. Although the lower degradation efficiency of TPHs (73%–89%) in the bioreactors containing both the FCM and ODB indicated a slight competition between them, the ODB were still effective in the presence of the FCM especially at greater concentrations of TPHs. Despite the fact that the addition of finished compost (FC) to the PS changed the microbial composition in the composting bioreactors; the microbial populations exhibited little variation during the process. This study indicated that the isolated ODB, alone or in the presence of the FCM, effectively degraded the PS in the composting process.

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**Abbreviations:** BHM, Bushnell–Haas medium; FC, finished compost; FCM, finished compost microorganisms; OD, optical density; ODB, oil degrading bacteria; PHs, petroleum hydrocarbons; PS, petroleum sludge; TPHs, total petroleum hydrocarbons

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<https://doi.org/10.1016/j.eti.2022.102319>

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## 1. Introduction

Production, storage, transport, processing and utilization of fossil fuels are among the main sources of environmental pollution by petroleum hydrocarbons. The processing of crude oil in oil refinery plants results in the production of petroleum sludge (PS). PS as a complex mixture contains different types of petroleum hydrocarbons (PHs) with varying degradability (Gielnik et al., 2019; Muangchinda et al., 2018). Different physical or chemical technologies such as photo-degradation, incineration, and adsorption have been used for remediation of PHs-contaminated media (Gupta and Gupta, 2015; Kumar and Gupta, 2020; Muangchinda et al., 2018). As a bioremediation strategy, composting process has been proposed as a promising and economical approach for the degradation of PHs (Gielnik et al., 2019; Muangchinda et al., 2018). In this framework, the application of bacterial consortia has been used to promote biodegradation efficacy due to the fact that the synergistic metabolic activities of various strains in the mixed culture are effective to enhance the degradation of PHs (Awasthi et al., 2018; Muangchinda et al., 2018). Therefore, the isolation and selection of bacterial strains with strong degradation ability and survival in a wide range of environmental conditions can be a key step in implementing the bioremediation of PHs.

The application of compost as an amendment or bulking agent has been shown to successfully support microbial activity and enhance hydrocarbons biodegradation due to higher microbial biomass and diversity (Gomez and Sartaj, 2014). In addition, compost provides a suitable micro-environment for mass transfer and hence, facilitates access of the microorganisms to PHs. Humic acids which are present in compost can also promote the desorption of PHs from PS and increase bioavailability (Kulikowska, 2016; Ren et al., 2018).

Other studies have however shown that indigenous compost microorganisms can interfere and affect the efficacy of bioaugmentation agents. Accordingly, adding of oil-degrading microorganisms as a bioremediation strategy remains still today one of the most controversial issues as some studies have reported possible competition between indigenous compost microorganisms and ODB (Awasthi et al., 2018; Li et al., 2019b; Tao et al., 2019). Some studies have shown that the abundance and enzymatic activities of added ODB are gradually outcompeted by the indigenous microorganisms which are more adapted to the local environmental conditions (Awasthi et al., 2018; Demichelis et al., 2017). Accordingly, there exists research interest in the effectiveness of bioaugmentation and its capacity to enhance PHs biodegradation (Jiang et al., 2016; Tao et al., 2019). In this regard, it would be interesting to monitor the microbial dynamics over the process duration to determine the mechanisms of bioremediation (Gielnik et al., 2019).

Considering the above, it is necessary to investigate the capability of ODB in co-existence and competition with finished compost microorganisms (FCM) in the composting process for bioremediation of PS. To the best of the authors' knowledge, the effect of PHs concentrations on the competition between ODB and FCM has not been reported in the literature. Therefore, the present study was conducted with the following objectives: (i) to isolate ODB from PS with high potential for PHs degradation; (ii) to assess the possible competition between inoculated ODB and FCM in the composting process; and (iii) to determine the effect of total petroleum hydrocarbons (TPHs) concentrations on the competition between ODB and FCM in the composting process for degradation of PHs.

## 2. Materials and methods

### 2.1. Petroleum sludge and finished compost

The PS sample was collected from an oil refinery plant located in Arak, Iran. The basic characteristics (dry weight basis) of the PS were as follows: TPHs level of 255.1 g/kg, moisture content of 27.6%, organic carbon concentration of 529 g/kg, phosphorus level of 1.0 g/kg, nitrogen content of 1.8 g/kg, and pH of 6.1. The finished compost (FC) as an amendment was obtained from a local composting facility in Arak. Then, it was sieved through < 2 mm in order to remove impurities and large particles. The PS and FC samples were transferred to a laboratory and stored in dark before use. In order to deactivate existing indigenous microorganisms, a portion of the FC and PS was autoclaved at 121 °C.

### 2.2. Isolation and identification of oil-degrading bacteria

To isolate ODB from the PS, 100 ml of Bushnell–Haas medium (BHM) was supplemented with the PS (5 g) and crude oil (1%) as carbon source. After shaking at 30 °C for 7 days, 5 ml of the incubated medium was inoculated in fresh BHM and incubated. After addition of 100 µl of the incubated culture to the surface of nutrient, the observed colonies were isolated. After overnight incubation at 37 °C, differential biochemical tests including gram staining citrate, catalase, urease, oxidase, H<sub>2</sub>S production, triple sugar iron, nitrate reduction, and indole production were performed to identify the colonies.

DNA was extracted from the bacterial colonies according to the QIAamp DNA mini kit manual (Qiagen, Valencia, CA). The extracted DNA was analyzed using a NanoDrop 2000 system (Thermo Scientific, Waltham, MA, USA). The 16SrDNA gene was amplified by polymerase chain reaction (PCR) according to the previously described procedure (Koolivand et al., 2019b).

**Table 1**  
Characteristics of the composting bioreactors used in the present study.

Composting bioreactors	TPHs concentrations (g/kg)	Conditions of bioreactors	Purpose of bioreactors
E <sub>1</sub> E <sub>2</sub>	10 30	Autoclaved PS + autoclaved FC + ODB inoculation	Determination of the ODB potential for TPHs degradation
E <sub>3</sub> E <sub>4</sub>	10 30	Non-autoclaved PS + autoclaved FC	Determination of the potential of all the native bacterial strains of PS for TPHs degradation
E <sub>5</sub> E <sub>6</sub>	10 30	Autoclaved PS + non-autoclaved FC	Determination of the FCM potential for TPHs degradation
E <sub>7</sub> E <sub>8</sub>	10 30	Autoclaved PS + non-autoclaved FC + ODB inoculation	Determination of the combined potential of the ODB and FCM for TPHs degradation so as to determine the ODB and FCM competition
E <sub>9</sub>	10	autoclaved PS	Control
E <sub>10</sub>	30	autoclaved PS	Control

### 2.3. Crude oil degradation in Bushnell–Haas medium

All the strains isolated from the PS were separately added to BHM and 1% crude oil to measure their abilities for growth and degradation of crude oil. The growth of the strains was measured using optical density at 600 nm (OD<sub>600nm</sub>) at the intervals of 2, 4, 7, 10, and 12 days. Out of 24 strains, 6 strains exhibiting the highest OD<sub>600nm</sub> and the degradation rate of crude oil were selected as ODB. Then, they were mixed in equal ratios to form a consortium of ODB to be used for the main experiments.

The effect of pH values (5.0–9.0) on the efficiency of the ODB in biodegrading crude oil was also examined. First, the bacterial consortium was added to the mixture of BHM and crude oil (1%) and then incubated at 30 °C under different pH values. At the end of incubation period (7 days), the biodegradation of crude oil and growth rate of the consortium were measured.

Moreover, the ability of the consortium for decomposition of multiple concentrations of crude oil (1, 2, 3, 4, and 5%) was determined in BHM. Briefly,  $1.5 \times 10^8$  CFU/ml of the ODB were transferred to 500 ml-capacity Erlenmeyer flasks and incubated at 30 °C with an initial pH of 7.0 and agitated at a speed of 120 rpm. After 7 days, the degradation rates of TPHs were determined. Control samples (without ODB addition) were also subjected to the same BHM experiments. The results obtained from BHM were used in designing the composting experiments in terms of initial levels of TPHs.

### 2.4. Experimental design and the operation of composting bioreactors

In order to determine the efficiency of the composting process in degrading PHs content of the PS, 10 polypropylene containers were used as composting bioreactors. As provided in Table 1, some of the composting bioreactors were bioaugmented with the ODB. The ODB was inoculated into the bioreactors with an initial amount of  $1.5 \times 10^8$  CFU/g of dry mixture. All the composting experiments were conducted in duplicate. Based on the results obtained from the BHM tests, the initial TPHs levels in the composting bioreactors were adjusted at 10 and 30 g/kg. The bioreactors were filled with the PS and mixed with different amounts of the FC with a total weight of 3 kg. The PS and FC mixture was completely mixed to obtain homogeneous distribution of PHs. Aeration of the bioreactors was supplied by means of oil-free pumps at the rate of 1 l/min.kg (Koolivand et al., 2019c). In addition, the content of the bioreactors was manually blended twice a week to guarantee sufficient oxygen and homogeneity. The C/N/P ratio was set at 100/5/1 (Koolivand et al., 2018) by adding mineral nutrients (K<sub>2</sub>HPO<sub>4</sub> and NH<sub>4</sub>Cl). The humidity was adjusted to 50%–55% through watering the samples twice a week.

### 2.5. Sampling procedure and quantification methods

Before sampling, the composting material was homogenized by mixing. Then, five samples each weighing 10 g were collected from each bioreactor at predetermined time intervals (weeks 0, 3, 6, 9, and 12). For each sampling, three sub-samples were selected from different depths, mixed and homogenized. The composite samples were kept at room temperature and dark conditions until analyzed.

The estimation of total microbial population in the samples was done using the serial dilution method. Briefly, one gram of the composting mixture was sampled and serially diluted in sterile distilled water and then, one ml from each dilution was transferred to plates. After incubation, enumeration of heterotrophs was performed by the plate counting and expressed as colony forming units (CFU) (Alef and Nannipieri, 1995; Awasthi et al., 2018; Wu et al., 2016). The obtained values were multiplied by the dilution factor and expressed in CFU/g of composting materials. In order to measure TPHs, the extraction procedure was based on TNRCC (2001) methods, with the use of mechanical shaking and n-pentane as a

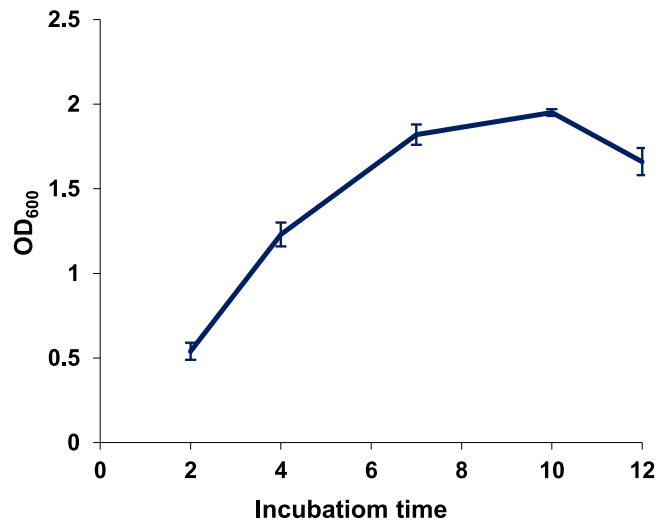


Fig. 1. Growth curve of the isolated ODB in BHM.

solvent. TPHs were quantified by gas chromatograph (GC) using a flame ionization detector (FID) (Shimadzu) following the procedure presented by Koolivand et al. (2013a,b). The reduction percentage of TPHs was based on the corresponding initial concentrations in each treatment. All the chemicals were acquired from Sigma-Aldrich Company and were of analytical grade. All the tests were conducted in duplicate.

## 2.6. Data analysis

Microsoft Excel and SPSS package were used to perform the statistical analyses. The computed results were shown as means  $\pm$  standard deviations. Significant differences among the treatments were assessed by one-way ANOVA ( $p < 0.05$ ). The sequences were analyzed by Chromas and aligned with Clustal X2.0. The similarity of the sequences in the NCBI database was searched by using BLAST.

## 3. Results and discussion

### 3.1. Characteristics of the oil-degrading bacteria

The results obtained from NCBI BLAST indicated that the isolated ODB were *Sphingomonas olei* strain KA1, *Acinetobacter radioresistens* strain KA2, *Enterobacter hormaechei* strain KA3, *Staphylococcus equorum* strain KA4, *Acinetobacter radioresistens* strain KA5, and *Enterobacter hormaechei* strain KA6. The sequences of the mentioned strains have been deposited in the NCBI under the accession numbers of MK127543, MK127544, MK127545, MK127546, MK127547, and MK127548, respectively (Abtahi et al., 2020; Koolivand et al., 2019b; Parhamfar et al., 2020). The related phylogenetic trees and the findings of the biochemical tests performed for the strains are available in our previously published papers (Koolivand et al., 2020; Poorsoleiman et al., 2020a,b).

### 3.2. Crude oil degradation in Bushnell–Haas medium

The OD<sub>600</sub>, as a measure of the ODB growth in the presence of crude oil, was examined in BHM. Fig. 1 indicates the growth curve of the ODB over the incubation periods of 2, 4, 7, 10, and 12 days. Based on the results, the ODB entered the logarithmic phase of growth after a period of 7–10 days. Accordingly, an incubation time of 7 days was chosen for all the BHM experiments.

The impact of pH on the ODB growth and crude oil (1% v/v concentration) degradation was evaluated as it is an important factor influencing the ODB metabolism and PHs solubility. As can be seen from Fig. 2, the highest growth of the ODB coincided with maximum biodegradation of crude oil in pH range of 6.0–8.0. For this pH range, 65%–72% of crude oil was degraded by the ODB over the incubation period (7 days). The cell growth and crude oil degradation elevated continuously as pH was increased from 5.0 and the highest values were attained at 7.0. Further increment in pH beyond the neutral range caused a drop in biomass formation and the corresponding biodegradation rate was in the range of 52%–56%. This can be mainly attributed to the intrinsic characteristics of bacteria in terms of tolerating extreme pH values. Furthermore, any fluctuation in pH beyond the optimal range can affect the solubility of PHs and thereby their availability to the ODB (Li et al., 2019a; Muangchinda et al., 2018). The results are in agreement with previous studies

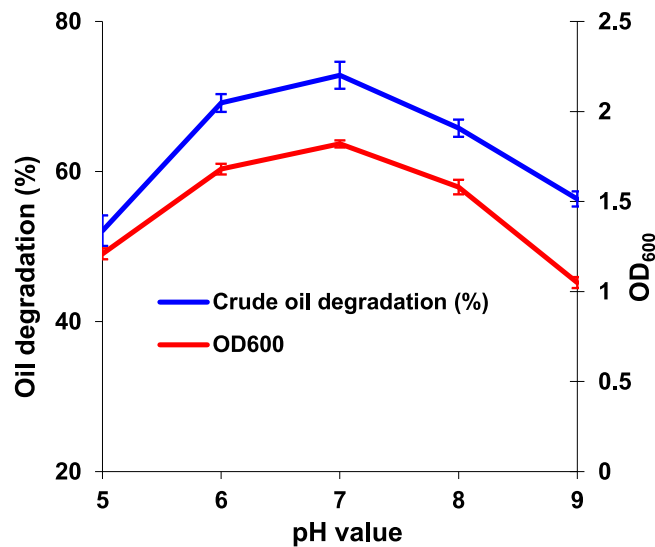


Fig. 2. Effect of pH on the biodegradation of crude oil in BHM after 7 days at 1% crude oil concentration.

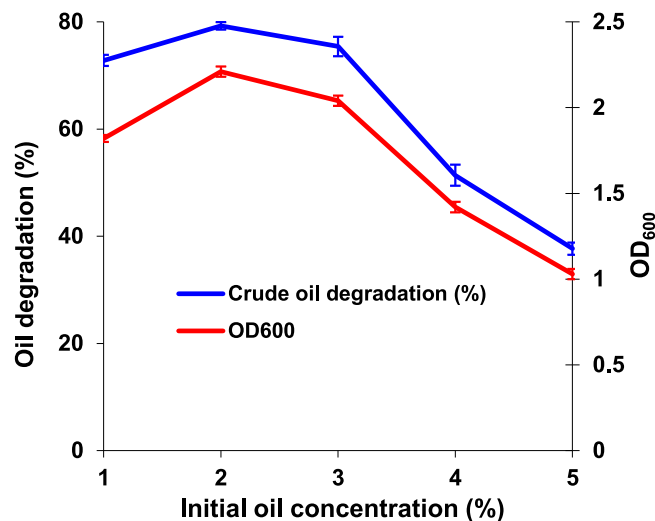


Fig. 3. Effect of initial concentration on the crude oil biodegradation in BHM after 7 days at pH 7.

(Muangchinda et al., 2018; Wang et al., 2016) showing ODB prefer a neutral pH to grow and degrade PHs. Thereupon, the composting process was performed at neutral condition without pH adjustment.

The concentration of TPHs should be optimized in the composting process to determine the level of PHs which can be efficiently degraded by the ODB. Accordingly, the ability of the ODB to degrade different levels (1%–5%) of crude oil was first investigated in BHM. As can be observed from Fig. 3, the ODB grew well at 1%–3% level of crude oil in BHM. At this range of concentration, 72%–75% of crude oil was degraded over the incubation time of 7 days. The high mineralization of these amounts of crude oil (1%–3%) is attributed to the inherent metabolic potential of the ODB and also to the synergistic effects between the isolated strains. Other studies (Kamyabi et al., 2017; Mnif et al., 2015) also indicated that the application of a mixture of ODB is more effective since each strain can metabolize specific types of PHs. However, greater initial crude oil levels (4 and 5%) resulted in reduced removal efficiencies since high amounts of PHs may be toxic to the ODB. Hence, the initial content of crude oil and TPHs are of utmost importance in conducting bioremediation methods such as composting process. The results obtained from BHM experiments indicated that 1%–3% concentration of crude oil was the optimal range for the degradation by the ODB. Therefore, the concentration of TPHs was adjusted at 10 and 30 g/kg in the composting bioreactors.

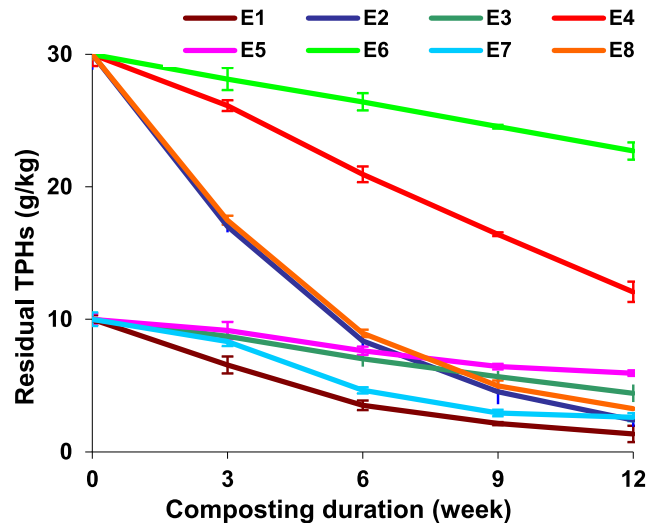


Fig. 4. Trend of TPHs degradation in the composting bioreactors over the process duration.

### 3.3. Biodegradation of the petroleum sludge in the composting bioreactors

The potential of the ODB to degrade the PS was examined in the composting bioreactors according to the BHM findings. In this regard, the TPHs levels were set at 10 and 30 g/kg by adjusting the PS/FC mixing ratio since it can largely influence the hydrocarbons degradation (Koolivand et al., 2013b, 2018). The pattern of TPHs dissipation in the composting bioreactors has been shown in Fig. 4. The percentages of TPHs removal in the bioreactors E<sub>1</sub>, E<sub>2</sub>, E<sub>3</sub>, E<sub>4</sub>, E<sub>5</sub>, E<sub>6</sub>, E<sub>7</sub>, and E<sub>8</sub> were computed to be 86.5, 92.0, 55.8, 59.8, 40.6, 24.3, 73.9, and 89.1%, respectively after 12 weeks. Table 2 shows the comparison of these findings with other similar studies (Alves et al., 2019; Becarelli et al., 2019; Escobar-Alvarado et al., 2018; Hur and Park, 2003; Hwang et al., 2001; Lin et al., 2012; Mihial et al., 2006; Namkoong et al., 2002; Paladino et al., 2016; Park et al., 2001). Based on the ANOVA results, the TPHs removals in the reactors E<sub>3</sub>, E<sub>4</sub>, E<sub>5</sub>, and E<sub>6</sub> exhibited significant differences ( $p < 0.05$ ) with E<sub>1</sub>, E<sub>2</sub>, E<sub>7</sub>, and E<sub>8</sub>. The very low amounts of TPHs removal in the control treatment E<sub>9</sub> (5.7%) and E<sub>10</sub> (6.1%) were an indication of the fact that the TPHs reduction was mainly due to the activities of microbial population existed in the composting bioreactors. Hence, non-biological mechanisms such as volatilization exhibited little effect in TPHs removal. The similar trends of TPHs reduction in the experiments containing the ODB (E<sub>1</sub>, E<sub>2</sub>, E<sub>3</sub>, E<sub>4</sub>, E<sub>7</sub>, and E<sub>8</sub>) indicated that the main fraction of PHs biodegradation occurred over the first 8 weeks of the process. From the week 8 onward, the rate of PHs decomposition dropped. This observation is in line with the previous studies (Koolivand et al., 2019a, 2014) reporting that the biodegradation of PHs was fast in the beginning of the process and then gradually declined with time. The reason for this downward pattern is the fact that easily-biodegradable hydrocarbons are rapidly degraded by microbial community. Hence, the residual hydrocarbons become recalcitrant to degradation (Ren et al., 2018; Robichaud et al., 2019). In addition, the metabolites generated during biodegradation may hinder PHs decomposition mainly due to the suppressive action on the microbial community (Chen et al., 2019; Pacwa-Płociniczak et al., 2019).

### 3.4. Competition between the oil degrading bacteria and finished compost microorganisms

Since several factors could affect PHs biodegradation in a bioremediation process, the results obtained from field-based methods such as composting may be different from laboratory findings (Awasthi et al., 2018; Robichaud et al., 2019). In this framework, a major factor is the potential competition between the ODB and FCM. The low efficacy of TPHs degradation in the bioreactors E<sub>5</sub> (40.6%) and E<sub>6</sub> (24.3%) confirmed the limited abilities of the FCM in degrading PHs, particularly at high concentrations of TPHs. On the other hand, the bioreactors E<sub>1</sub> and E<sub>2</sub> merely containing the ODB exhibited the highest removal rates of TPHs. This indicates that the ODB can effectively degrade PHs when there are no other microorganisms in the composting mixture. However, TPHs removals dropped in the bioreactors E<sub>7</sub> and E<sub>8</sub> experiencing simultaneous applications of the ODB and FCM. This could be because there has been a competition between the ODB and FCM. These results are in line with another study conducted by Tao et al. (2019) reporting a competition between inoculated bacteria and indigenous microbial community. This may be due to the competition for growth and consumption of carbon and energy sources. Moreover, some native microorganisms such as protozoa could prey the inoculated bacteria (Ren et al., 2018). As a result of this competition, the inoculated ODB is not highly effective in the presence of the FCM. The important point is that the TPHs removal in the treatment E<sub>8</sub> (89.1%) was higher than that in the E<sub>7</sub> (73.9%). The reason is the higher concentration of TPHs in the E<sub>8</sub> which can limit the growth of the FCM and thereby

**Table 2**  
Comparison of removal efficiencies of composting remediation for PHs.

Sludge (soil) TPHs concentration (g/kg)	Sludge (soil)/compost (dry wt.)	Compost material	Duration (day)	Removal efficiency (%)	References
31.8	2/1	Yard trimmings, cactus	140	66	(Escobar-Alvarado et al., 2018)
54	3/1	Wood chips	60	47.6	(Becarelli et al., 2019)
39.1	1/1	Grass clippings, fertilizer mixture	181	71.9–96.7	(Mihial et al., 2006)
40.3	–	Sewage sludge, fish sludge	20	23.9	(Alves et al., 2019)
10	1/0.5	Sewage sludge, mature compost	30	98.4	(Namkoong et al., 2002)
10	1/0.1–1/1	Sewage sludge, yard waste	30	69.3–99.6	(Park et al., 2001)
10.7	1/0.5	Sewage sludge	30	98.1	(Hur and Park, 2003)
10	1/0.1–1/1	Sewage sludge, mature compost	30	73.8–95.3	(Hwang et al., 2001)
26.3	1/1	Food waste, mature compost	30	92.4	(Lin et al., 2012)
21.8	1/0.4	Fresh organic waste	151	82	(Paladino et al., 2016)
255	1/7.7–1/26.6	mature compost	84	86–92	Present study

**Table 3**  
Percentages of TPHs removal in the composting bioreactors over the process duration.

Process duration	TPHs removal (%)							
	E <sub>1</sub>	E <sub>2</sub>	E <sub>3</sub>	E <sub>4</sub>	E <sub>5</sub>	E <sub>6</sub>	E <sub>7</sub>	E <sub>8</sub>
Week 0	0	0	0	0	0	0	0	0
Week 3	34.40	43.28	12.85	12.90	8.50	6.20	16.85	41.70
Week 6	30.60	28.85	17.05	17.27	15.20	5.73	36.75	28.47
Week 9	13.70	12.73	13.45	15.10	11.95	6.23	17.10	13.27
Week 12	7.80	7.17	12.45	14.50	4.95	6.13	3.20	5.70
Total	86.50	92.03	55.80	59.77	40.60	24.30	73.90	89.13

reduce the competition between the ODB and FCM. The lower efficiency of TPHs removals in the reactors E<sub>3</sub> and E<sub>4</sub> in comparison with E<sub>1</sub> and E<sub>2</sub> indicated a limited competition between the ODB and other microbial population present in the PS.

The percentage of TPHs removal over the sampling time has been shown in Table 3. The comparison of the removal efficiency in the bioreactor E<sub>7</sub> with those in the E<sub>1</sub> and E<sub>8</sub> reveals important points regarding the competition between the ODB and FCM. The lower efficiency of the bioreactor E<sub>7</sub> containing both the ODB and FCM compared with E<sub>1</sub> containing only the ODB verifies their competition particularly over the first 3 weeks of the process. Thus, in the presence of the FCM, the capability of the ODB for PHs biodegradation is limited at the beginning of the process. From this week onward, the degradation of TPHs in these two reactors became almost similar. This indicated that the ODB can effectively survive in the presence of the FCM after week 3. The better performance of the bioreactor E<sub>8</sub> in comparison with E<sub>7</sub> also demonstrates the mentioned competition since the higher concentration of TPHs in the E<sub>8</sub> (30 g/kg) can limit the growth and activities of the FCM. The detailed descriptions of this competition in terms of composition of microbial communities over the process duration have been provided in Section 3.5.

### 3.5. Composition of microbial communities in the composting bioreactors

Fig. 5 provides the numbers of heterotrophs (Log CFU/g) of composting mixture in each bioreactor over the process duration. Except for the bioreactors E<sub>3</sub> and E<sub>4</sub>, the initial number of heterotrophs was greater than 10<sup>6</sup> CFU/g. It has been reported that the minimum density of microorganisms in the bioremediation processes should be 10<sup>5</sup> CFU/g (Hinchee et al., 1995). The microbial density counted in the bioreactors was in good agreement with other computed values in the literature. In the bioremediation of diesel-contaminated soil amended with compost, Nwankwegu et al. (2016) reported counts greater than 10<sup>7</sup> CFU/g of heterotrophs. The number of heterotrophs observed by Dadrasnia and Agamuthu (2013) and Agarry et al. (2015) were around 10<sup>8</sup> CFU/g in oil-contaminated soil composting. Babaei et al. (2020) also reported that the highest number of heterotrophs in the composting of oil-based drilling cuttings by bagasse were more than 10<sup>7</sup> CFU/g. A minor downward trend observed for heterotrophs over the process duration can be explained by the relatively lower amount of PHs required for growth. Moreover, the application of strains in new environments has some limitations resulted mainly from low adaptability (Awasthi et al., 2018).

In the presence of PHs, the growth of microbial decomposers could alter the composition of the bacterial community (Galitskaya et al., 2021). Table 4 provides the microbial population during the degradation of PHs in the bioreactors.

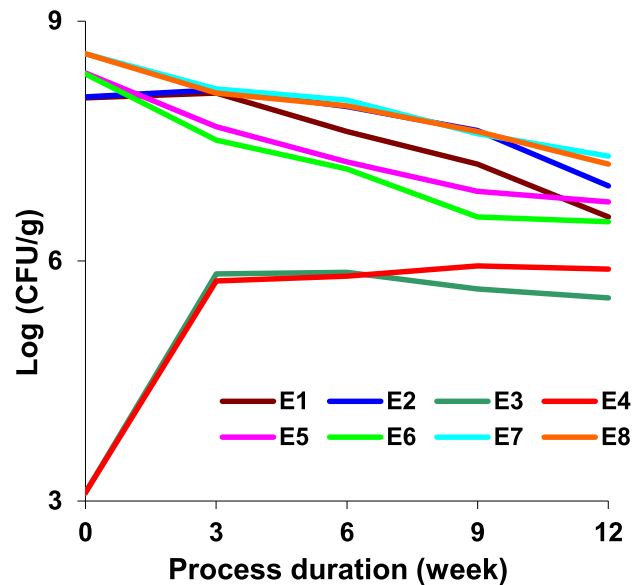


Fig. 5. Density of heterotrophs in the composting bioreactors over the process duration.

It comes as no surprise that the addition of the FC to the PS would have some effect on the bacterial diversity in the composting reactors. Except for the inoculated ODB, *Actinomyces* sp., *Bacillus* sp., *Corynebacterium* sp., *Aspergillus* sp., *Penicillium* sp., and *Mucor* sp. were naturally observed in the treatments containing non-autoclaved FC. In line with our results, it has been reported in the literature that microbial species like *Bacillus* sp., *Actinomyces* sp., *Aspergillus* sp., *Penicillium* sp., and *Mucor* sp. are the dominant species in the composting of oil contaminated environments (Al-Kindi and Abed, 2016; Babaei et al., 2020; Tran et al., 2020). However, unlike the results obtained by Robichaud et al. (2019), our findings did not indicate a significant change in the microbial populations over the process duration.

On the other hand, in accordance with Awasthi et al. (2018) we also observed that the microbial communities were different between the various bioreactors. This difference can be mainly attributed to the addition of the FC containing some genera which may not be bacteria directly responsible for PHs degradation. It has been stated that these genera may promote PHs biodegradation pathways and therefore FCM and ODB could accelerate degradation through synergistic actions (Ren et al., 2018). Moreover, Chen et al. (2019) reported that the addition of aged refuse was beneficial for increasing ODB density and hence contributed to the high TPHs degradation in the soil. However, the results of the current research showed that in spite of the effect of the FCM in increasing total microbial density, it resulted in inactivating some species of the isolated ODB (*Sphingomonas* sp. and *Staphylococcus* sp.) and a slight decrease in the overall removal of TPHs due to the ODB and the FCM competition. Nevertheless, the important point here is that the remaining ODB (*Acinetobacter* sp. and *Enterobacter* sp.) can still effectively degrade PHs in the presence of the FCM, particularly when the concentration of PHs is high. Further work should be conducted in the future to examine the detailed role of this competition.

### 3.6. Kinetic models of petroleum hydrocarbons biodegradation in the composting bioreactors

The kinetic of TPHs removal was investigated so as to reach a better understanding of PHs degradation over the composting process. In this regard, the first (Eqs. (1) and (2)) and second-order kinetics (Eqs. (3) and (4)) were used as follows:

$$C_t = C_p e^{-k_1 t} \quad (1)$$

$$t_{1/2} = \ln 2 / k_1 = 0.693 / k_1 \quad (2)$$

$$1/C_t = k_2 t + (1/C_p) \quad (3)$$

$$t_{1/2} = 1 / k_2 C_p \quad (4)$$

Where  $C_p$  is the primary concentration of TPHs (g/kg),  $t_{1/2}$  is the time (d) taken for 50% degradation of PHs,  $C_t$  is the concentration of TPHs (g/kg) at time  $t$ ,  $k_1$  ( $d^{-1}$ ) and  $k_2$  (g/kg.d) are the biodegradation rate constants for the first- and second-order kinetics, respectively.

The computed data presented in Table 5 indicated that the pattern of PHs biodegradation is similar to the first-order kinetics. The values of  $k_1$  calculated for the bioreactors E<sub>1</sub>–E<sub>8</sub> were in the range of 0.023–0.213  $d^{-1}$  which were different from those computed by He et al. (2019) (0.0003–0.0049  $d^{-1}$ ) and Gomez and Sartaj (2013) (0.004–0.043  $d^{-1}$ ). The values



**Table 4**  
Composition of microbial population in the composting bioreactors over the process duration.

Process duration	E <sub>1</sub>	E <sub>2</sub>	E <sub>3</sub>	E <sub>4</sub>	E <sub>5</sub>	E <sub>6</sub>	E <sub>7</sub>	E <sub>8</sub>
Week 0	<i>Sphingomonas</i> sp. <i>Acinetobacter</i> sp. <i>Enterobacter</i> sp. <i>Staphylococcus</i> sp.	<i>Sphingomonas</i> sp. <i>Acinetobacter</i> sp. <i>Enterobacter</i> sp. <i>Staphylococcus</i> sp.	<i>Sphingomonas</i> sp. <i>Acinetobacter</i> sp. <i>Enterobacter</i> sp. <i>Staphylococcus</i> sp.	<i>Sphingomonas</i> sp. <i>Acinetobacter</i> sp. <i>Enterobacter</i> sp. <i>Staphylococcus</i> sp.	<i>Aspergillus</i> sp. <i>Penicillium</i> sp. <i>Mucor</i> sp. <i>Actinomyces</i> sp. <i>Bacillus</i> sp. <i>Corynebacterium</i> sp. <i>Acinetobacter</i> sp.	<i>Aspergillus</i> sp. <i>Penicillium</i> sp. <i>Mucor</i> sp. <i>Actinomyces</i> sp. <i>Bacillus</i> sp. <i>Corynebacterium</i> sp. <i>Acinetobacter</i> sp.	<i>Aspergillus</i> sp. <i>Penicillium</i> sp. <i>Mucor</i> sp. <i>Actinomyces</i> sp. <i>Bacillus</i> sp. <i>Corynebacterium</i> sp. <i>Sphingomonas</i> sp. <i>Acinetobacter</i> sp. <i>Enterobacter</i> sp. <i>Staphylococcus</i> sp.	<i>Aspergillus</i> sp. <i>Penicillium</i> sp. <i>Mucor</i> sp. <i>Actinomyces</i> sp. <i>Bacillus</i> sp. <i>Corynebacterium</i> sp. <i>Sphingomonas</i> sp. <i>Acinetobacter</i> sp. <i>Enterobacter</i> sp. <i>Staphylococcus</i> sp.
Week 3	Same as week 0	Same as week 0	Same as week 0	Same as week 0	Same as week 0	Same as week 0	<i>Aspergillus</i> sp. <i>Penicillium</i> sp. <i>Mucor</i> sp. <i>Actinomyces</i> sp. <i>Bacillus</i> sp. <i>Corynebacterium</i> sp. <i>Acinetobacter</i> sp. <i>Enterobacter</i> sp.	<i>Aspergillus</i> sp. <i>Penicillium</i> sp. <i>Mucor</i> sp. <i>Actinomyces</i> sp. <i>Bacillus</i> sp. <i>Corynebacterium</i> sp. <i>Acinetobacter</i> sp. <i>Enterobacter</i> sp.
Week 6	Same as week 0	Same as week 0	Same as week 0	Same as week 0	Same as week 0	Same as week 0	Same as week 3	Same as week 3
Week 9	Same as week 0	Same as week 0	Same as week 0	Same as week 0	Same as week 0	Same as week 0	Same as week 3	Same as week 3
Week 12	Same as week 0	Same as week 0	Same as week 0	Same as week 0	Same as week 0	Same as week 0	Same as week 3	Same as week 3

**Table 5**  
Kinetic constants computed for TPHs degradation in the composting bioreactors.

Composting bioreactors	First-order kinetics			Second-order kinetics		
	$k_1$	$t_{1/2}$ (d)	$R_1^2$	$k_2$	$t_{1/2}$ (d)	$R_2^2$
E <sub>1</sub>	0.171	4.05	0.997	0.053	1.89	0.094
E <sub>2</sub>	0.213	3.25	0.999	0.031	1.08	0.887
E <sub>3</sub>	0.069	10.04	0.991	0.011	9.09	0.960
E <sub>4</sub>	0.076	9.12	0.982	0.004	8.33	0.934
E <sub>5</sub>	0.047	14.74	0.983	0.006	16.67	0.981
E <sub>6</sub>	0.023	30.13	0.998	0.001	33.33	0.992
E <sub>7</sub>	0.124	5.59	0.958	0.023	4.35	0.954
E <sub>8</sub>	0.190	3.65	0.995	0.023	1.45	0.944

of the computed  $t_{1/2}$  are also not in accordance with those reported by [Nwankwegu et al. \(2016\)](#) (17–34 days) and [Babaei et al. \(2020\)](#) (37–79 days). The reason is that numerous factors such as the nature of PHs, TPHs concentration, the method of bioremediation, and the characteristics of ODB can affect the rate constant of PHs biodegradation ([He et al., 2014](#); [Kulikowska, 2016](#)). The higher  $k_1$  and lower  $t_{1/2}$  observed in the bioreactor E<sub>1</sub> and E<sub>2</sub> compared to the corresponding values in the E<sub>7</sub> and E<sub>8</sub> also verified possible competition between the ODB and the FCM.

#### 4. Conclusions

The ability of the isolated ODB in PHs degradation both in BHM and composting process as well as in competition with FCM during PS bioremediation was studied. The ODB degraded 72%–75% of crude oil (1%–3% concentration) in BHM after 7 days. In the composting reactors, 86%–92% of TPHs was removed by the ODB after 12 weeks. Although the addition of the FC to the PS affects the bacterial diversity in the composting bioreactors, little change in the bacterial populations was observed over the process duration. The slight decrement in the ODB efficiency in the presence of the FCM verified a competition between the ODB and FCM. However, this competition is not significant enough to prevent the effective degradation of PHs by the ODB. Hence, the PS can be effectively remediated by the isolated ODB in the composting process.

#### CRedit authorship contribution statement

**Ali Koolivand:** Conceptualization, Methodology, Supervision, Writing – review & editing. **Hamid Abtahi:** Methodology, Supervision, Writing – review & editing. **Maryam Parhamfar:** Methodology, Writing – review & editing. **Reza Saeedi:** Methodology, Writing – review & editing. **Frederic Coulon:** Writing – review & editing. **Vinod Kumar:** Writing – review & editing. **José Villaseñor:** Writing – review & editing. **Majid Sartaj:** Writing – review & editing. **Niloofar Najarian:** Methodology, Writing – review & editing. **Maedeh Shahsavari:** Formal analysis, Writing – review & editing. **Paria Seyedmoradi:** Formal analysis, Writing – review & editing. **Leila Rahimi:** Formal analysis, Writing – review & editing. **Fatemeh Bagheri:** Formal analysis, Writing – review & editing.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Acknowledgments

The authors would like to acknowledge the financial support from Arak University of Medical Sciences (Grant No. 2819, 3490, and 3491).

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